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PRECLINICAL STUDY

Dynamics of lymphatic regeneration and flow patterns after lymph node dissection

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Abstract Knowledge about the mechanisms of regeneration of the lymphatic vasculature after surgical trauma is essential for the development of strategies for the prevention and therapy of lymphedema. However, little is known about the alterations of lymphatic flow directly after surgical trauma. We investigated lymphatic function in mice using near-infrared imaging for a period of 4 weeks after surgeries that mimic sentinel lymph node biopsy (SLNB) or axillary lymph node dissection (ALND), by removal of the popliteal lymph node (LN) alone or together with the popliteal fat pad, respectively. SLNB-like surgery did not cause changes in lymphatic drainage in the majority of cases. In contrast, lymphatic drainage impairment shown by collecting vessel rupture, dermal backflow and rerouting of lymph flow via collateral vessels were observed after ALND-like surgery. All collateral vessels drained to the inguinal LN. These results indicate that less invasive surgery prevents lymphatic decompensation. They also reveal the development and maturation of collateral lymphatic vessels after extensive surgical trauma, which reroute the flow of lymph towards a different LN. These findings might be helpful for the development of strategies to prevent and/or treat post-surgical lymphedema.

Keywords Lymphatic flow · Sentinel lymph node · In vivo imaging · Vascular development

Abbreviations

ALND	Axillary lymph node dissection
DB	Dermal backflow
LV	Lymphatic vessel
LN	Lymph node
SLNB	Sentinel lymph node biopsy
MLD	Manual lymphatic drainage

Introduction

Understanding the mechanisms of regeneration of the lymphatic vasculature after surgical trauma is essential for the development of strategies for the prevention and therapy of lymphedema. Several studies have shown an increased risk for patients with axillary lymph node dissection (ALND) to develop post-therapeutic lymphedema, compared to patients after sentinel lymph node biopsy (SLNB) [1, 2]. It is obvious that SLNB results in less tissue damage compared to ALND, however there are no studies investigating lymph flow changes directly after trauma or studies that directly compare lymph flow changes after those two procedures. Common findings of patients that have been diagnosed with lymphedema are dermal backflow (DB), which describes a reverse flow of lymph fluid from the collecting vessels via precollecting vessels to dermal capillaries [3]. Moreover, rerouting of lymphatic drainage via collateral vessels and vessel rupture have been described in patients with established lymphedemas [4–6].

Until recently, most functional studies of the human lymphatic system have employed lymphoscintigraphy [4].

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The establishment of near-infrared (NIR) imaging methods of the lymphatic system has opened up new horizons by studying both the morphology of lymphatic vessels in addition to lymphatic function with one imaging modality [7]. NIR imaging also enables repetitive and longitudinal imaging, since there is no ionizing radiation needed for visualization of the lymphatic vasculature.

The aim of the present study was to investigate the changes of lymphatic flow after different types of surgical trauma. Using longitudinal NIR imaging of lymphatic flow over 4 weeks after surgery, mimicking either SLNB or ALND, we found that lymphatic drainage was minimally affected after SLNB-like surgery, whereas rupture of collecting vessels, DB and collateral vessel development were frequently observed after ALND-like surgery. The collateral vessels matured and rerouted the lymph flow towards a different LN. These findings underline the need for less traumatic surgery to prevent the development of post-surgical lymphedema, and they might also be helpful for the development of new conservative, surgical or pharmacological strategies by stimulation of collateral vessel development and/or restoration of lymphatic flow in collecting vessels.

Materials and methods

Animals

Male FVB mice ($n = 7$, 14 weeks age) were kept under conventional-specific pathogen free conditions and were provided water ad libitum and access to chow diet. The mean weight was 30.4 ± 1.5 g. The experiments were performed in accordance with animal protocols approved by the Kantonales Veterinäramt Zürich.

Surgery

Due to the anatomical similarities with the axillary region in humans, which is a bottleneck for the drainage of lymph fluid from the arm, we removed the popliteal lymph node (popLN) in the hind leg of mice, which receives lymph fluid from the entire lateral and most of the ventral and dorsal side of the lower leg [8]. Surgery was performed under i.p. anaesthesia via injection of 0.2 mg/kg medetomidine and 80 mg/kg ketamine. After depilation and skin disinfection, two different surgical procedures were performed. SLNB was mimicked by minimal-invasive removal of the popLN in the hind legs (group A, $n = 5$, Table 1). Extensive resection of a LN-region with removal of all tissue up to the muscle fascia (ALND-like surgery), was mimicked by removing the popliteal LN together with the popliteal fat pad in the hind legs ($n = 5$; group B, Table 1). Both legs of each mouse were treated to exclude side-dependent drainage effects. To rule out changes of lymphatic flow by repetitive NIR imaging, untreated legs ($n = 4$) were used as controls (group C, Table 1). The surgery in groups A and B lasted 20–30 min. Wounds were closed with absorbable filament (6-0 Prolene, Ethicon, Norderstedt, Germany). Metamizol was added to the drinking water for 24 h after surgery (8 drops/500 mL water; Ratiopharm GmbH, Ulm, Germany). Post-surgical wound healing and behaviour of the mice was checked regularly.

Synthesis of PEG-IRDye

The NIR dye conjugate **P40-D680** was obtained as previously described [9]. In brief, methoxypoly (ethylene glycol) (PEG) amine **P40** (40 kDa, 75 nmol, JenKem Technology USA Inc., Allen, TX) predissolved in 500 µL anhydrous DMSO was reacted with an equimolar amount of IRDye® 680LT NHS Ester (**D680**, LI-COR Biosciences, Lincoln, NE) at room temperature for 6 h. The crude reaction was freeze-dried overnight, reconstituted with 250 µL of HEPES buffered saline (HEPES 20 mmol/L, NaCl 142 mmol/L, pH 7.4) and purified by means of Fluorescent Dye Removal columns (Thermo Fisher Scientific, Rockford, IL). The purity of the recovered compound was controlled by high-performance liquid chromatography and the efficiency of the coupling procedure was assessed by UV absorption in methanol [9].

NIR lymphatic imaging

Mice were imaged 5 days prior surgery and weekly after surgery over a period of 4 weeks using NIR stereomicroscopy [10]. A StereoLumar.V12 stereomicroscope (Carl

Table 1 Overview of study groups and different interventions in the mouse legs

Mouse	Left leg	Right leg
1	SLNB-like surgery (group A)	ALND-like surgery (group B)
2	SLNB-like surgery (group A)	ALND-like surgery (group B)
3	SLNB-like surgery (group A)	ALND-like surgery (group B)
4	No surgery (group C)	SLNB-like surgery (group A)
5	No surgery (group C)	SLNB-like surgery (group A)
6	ALND-like surgery (group B)	No surgery (group C)
7	ALND-like surgery (group B)	No surgery (group C)

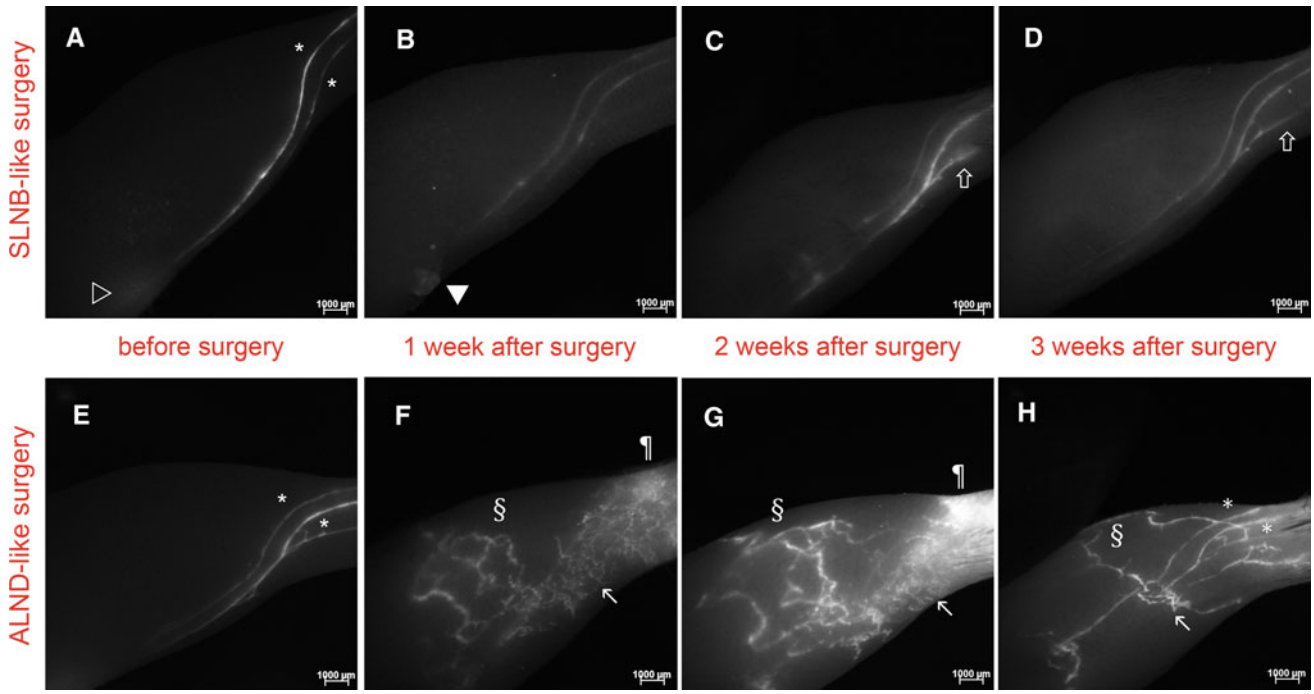

Fig. 1 Changes of lymphatic flow after SLNB-like and ALND-like surgery, visualized with NIR imaging. **a** NIR images of the lymphatic vessels of the right hind leg before SLNB-like surgery of the popliteal LN (popLN, *open right faced traingle*). Regular presentation of the lymphatic vasculature of the leg: two collecting vessels (*asterisk*) draining the dye to the popliteal LN before surgery. **b** Almost unaffected lymphatic drainage pathway 1 week after SLNB-like surgery. Cutaneous suture in the popliteal fossa (*filled inverted traingle*). Similar lymphatic drainage pathways are detected 2 (**c**) and 3 (**d**) weeks after SLNB-like surgery. The visualization of a third (*upwards white arrow*), more dorsal collecting vessel can sometimes be seen in the right hind limb, dependent on the injection pressure and injection site. **e** Pre-surgical imaging of the lymphatic vessels of the right hind limb before ALND-like surgery. **f** One week after ALND-like surgery, the dye-enriched lymph is rerouted and drained from the collecting vessels (*asterisk*) via dermal capillaries (*north west arrow*) and collateral LVs (*section sign*) towards the inguinal LN (not shown). Ruptured collecting vessel in the ankle region (*pilcrow sign*). **g** Two weeks after ALND-like surgery, increased numbers of collateral LVs (*section sign*) drain the lymph from DB areas (*north west arrow*). Leaked-out dye from rupture of the collecting vessel (*pilcrow sign*). **h** Reorganised lymphatic flow 3 weeks after surgery, with collateral LVs (*section sign*) draining directly from the collecting vessels (*asterisk*) and from dermal capillaries (*north west arrow*) to the ingLN (not shown). Residual DB is visualized at the area where the collecting vessels are interrupted (*north west arrow*)

Zeiss, Oberkochen, Germany) with AxioVision software (Zeiss) was adapted for far-red visualization by installation of a cooled EMCCD camera (Evolve eXcelon, Photometrics, Tuscon, AZ) with enhanced sensitivity to the near infrared spectrum, a high-powered light emitting diode (LED) system with illumination at 635 nm (CoolLED, Andover, UK) and specific filters for Cy5 wavelengths (Zeiss). Mice were anesthetized via i.p. injection as described above and placed in a supine position on a heating pad (38 °C). The leg was depilated and the foot was elevated to achieve a horizontal position of the leg. The contrast agent (5 µL of 25 µM P40D680) was injected intradermal between the 4th and the 5th toe of the left hind limb. Videos during the dye-injection, pulsing of the collecting vessels and an overview of the lymphatic vessels of the whole leg were taken. Analyses of the recorded videos were performed with ImageJ software [11]. Three parameters were monitored over time: rupture of collecting vessels, occurrence and dynamics of DB and the development of new, collateral vessels. These parameters were defined by findings in lymphedema patients of the literature [6]. Rupture of collecting vessel meant a circumscribed dye accumulation from the collecting LV (i.e. north east arrow in Fig. 2a). Collateral LVs were defined as LV draining towards other LN-regions, in this study the inguinal LN.

Results

The extent of trauma influences the disturbance of lymphatic flow

Mild swelling of the hind leg was noticed in all mice 2–3 days after surgery, but no wound infection or delayed wound healing was detected. Wound healing was completed 7–10 days after surgery. Longitudinal, non-invasive NIR imaging of the lymphatic vasculature of the hind legs showed major differences in flow patterns between the legs

with SLNB-like surgery (group A) and with ALND-like surgery (group B) (Fig. 1). Repetitive NIR imaging of the lymphatic vasculature revealed no changes or abnormal patterns of lymphatic flow in the legs of the control group C over the whole study period (Tables 2, 3, 4).

One week after SLNB surgery, normal drainage pathways of the dye-enriched lymph were seen via the collecting vessels towards the region of the excised LN in four out of five legs of this group (Fig. 1b). Lymphoceles developed at the former site of the LN in these legs. A rupture of the dorsal collecting vessel with adjacent DB was detected at the lateral side of the ankle in one case (Tables 2, 3). Two weeks after surgery collateral LVs were seen draining from the area of DB in this leg. The drainage pathways of the other four legs of group A remained normal. Similar drainage pathways were seen 3 weeks after surgery in group A (Tables 2, 3, 4). Four weeks after SLNB-like surgery a connection of the former afferent and efferent LV in popliteal region was visualized in the four legs with normal lymphatic drainage pathways (data not shown). DB with collateral LVs draining to the inguinal LN persisted in one leg of group A (Table 4).

One week after ALND surgery, rupture of the collecting vessels was seen in all legs and superficial collateral LVs were found in four out of five legs (Tables 2, 4; Fig. 1f). The collateral LVs originated either directly from areas of collecting vessel rupture or from dermal backflow areas (Fig. 1f). The subcutaneous localization was determined by dissection (data not shown). Two weeks after ALND surgery, DB was found in all legs and collateral LVs were detected in four out of five legs (Tables 3, 4). Rupture of collecting vessels was found in three legs (Table 2). The amount of legs with DB decreased to three out of five legs three weeks after ALND surgery (Table 3). A rupture of collecting vessels was found only in two legs at this time point (Table 2). Collateral vessels draining to the inguinal LN were found in 4 legs. The amount and density of collateral LVs in each leg increased from 2 to 3 weeks after surgery (Fig. 1g, h). Regular morphology of the collecting LV (without rupture) was found in all legs four weeks after ALND-like surgery although persistent DB was found in 2 out of 5 legs. The lymph was drained via collateral LVs to the inguinal LN in all legs (Table 4).

Table 2 Dynamics of collecting LV rupture 1–4 weeks after surgical trauma

	LN removal Group A	LN and fat pad removal Group B	No surgery Group C
1 week after surgery	1/5	5/5	0/4
2 weeks after surgery	0/5	3/5	0/4
3 weeks after surgery	0/5	2/5	0/4
4 weeks after surgery	0/5	0/5	0/4

Table 3 Dynamics of dermal backflow (DB) over a period of 4-weeks after surgical intervention

	LN removal Group A	LN and fat pad removal Group B	No surgery Group C
1 week after surgery	1/5	4/5	0/4
2 weeks after surgery	1/5	5/5	0/4
3 weeks after surgery	1/5	3/5	0/4
4 weeks after surgery	1/5	2/5	0/4

Table 4 Detection of collateral lymph vessels draining lymph to the inguinal LN over a period of four weeks after surgical intervention

	LN removal Group A	LN and fat pad removal Group B	No surgery Group C
1 week after surgery	0/5	4/5 (2/5)	0/4
2 weeks after surgery	1/5 (0/5)	4/5 (4/5)	0/4
3 weeks after surgery	1/5 (1/5)	4/5 (4/5)	0/4
4 weeks after surgery	1/5 (1/5)	5/5 (5/5)	0/4

The number of investigated legs with detection of pulsing of collateral vessels is displayed in parentheses

Collateral LVs develop, drain to the inguinal LN and take over the function of collecting vessels after ALND-like surgery

As described above, collateral LV development was found in all legs after ALND-like surgery and in one out of five legs after SLNB-like surgery. The morphology changed and the amount of collateral LVs increased over the period of three weeks after surgery, shown in Fig. 2. The maturation process involved changes of vessel calibre, flow velocity and pulsing activity. One week after surgery, the calibre of the newly visualized collateral vessels was irregular with constricted and dilated areas (Fig. 2a). The flow was slower in the collateral LV compared to the collecting vessels, evidenced by a slower decrease of signal intensity over time in the collateral compared to collecting vessels (Online Resource 1). Pulsing of collateral LVs was detected in 50 % of the cases one week after surgery (Table 4). Pulsing movement was only detected in the proximal third of the collateral LV (in proximity of the inguinal LN). Areas with pulsing activity increased during subsequent imaging sessions and pulsing throughout the entire collateral LV was visualized 3 weeks after surgery (Online Resources 2, 3). Increasing pulsing activity was accompanied by morphological changes of collateral LVs. The formerly irregular vessel calibre with constricted and

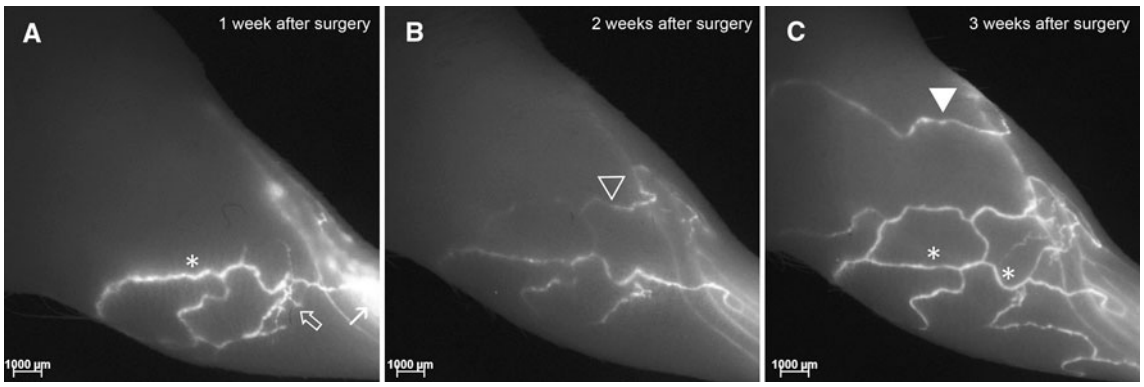

Fig. 2 Development and maturation of collateral LVs after ALND-like surgery, monitored with NIR imaging. **a** After 1 week, one main collateral LV (*asterisk*) drains dye-enriched lymph from an area of interstitial dye accumulation (*north east arrow*; due to burst of the dorsal collecting vessel) to the ingLN (not shown). Irregular calibre of this collateral LV with constricted and dilated segments. Small branches (*upwards white arrow*) are arising from the collateral LV and re-enter into the collateral vessel further downstream. **b** Two weeks after surgery, new collateral LVs (*open right faced traingle*) are visualized draining from the interrupted collecting vessel leading to an expansion of the collateral LV network. The inhomogeneous intravascular distribution of the dye indicates disturbances of lymphatic drainage in the collateral vessels. **c** Expansion of the collateral network by development of new collateral LVs rerouting the lymph from the interrupted collecting vessel to the ing LN (not shown) three weeks after ALND-like surgery. The calibre of the newly detected collateral LVs (*filled inverted traingle*) is irregular, whereas the vessel contour of already 'matured' collateral vessels is regular (*asterisk*)

dilated sections (Fig. 2a) matured to a vessel with more regular calibre throughout its length (Fig. 2c) 3 weeks after surgery. These 'matured' collateral LVs showed comparable flow to collecting LVs, with a fast decrease in signal intensity over time, together with high pulsing activity (Online Resource 3). In addition to the maturation, the amount of collateral LVs increased from 1 to 3 weeks after surgery forming a dermal lymphatic network to reroute the lymph via the collateral LVs towards the inguinal LN (Fig. 2a–c). Similar flow pathways were seen 4 weeks after surgery (data not shown).

Discussion

Understanding the pathophysiology of lymphedema is fundamental to prevent lymphedema development after surgery and to develop effective therapies for patients with chronic lymphedema. Using longitudinal NIR imaging of the lymphatic vasculature, we found that SLNB-like surgery did not alter the lymphatic drainage pathways in the majority of legs over a period of 4 weeks after surgery. In contrast, rupture of collecting vessels, DB and collateral LV development were commonly observed during the first 4 weeks after ALND-like surgery. Although we only imaged acute/subacute changes of lymphatic flow after surgery in the current study, the results might explain the lower incidence of lymphedema development after SLNB that has been reported in breast cancer patients [1, 2]. As seen after ALND-like surgery, the lymphatic system reacts to fluid overload due to downstream surgical trauma by rupture of LVs, DB and the development of collateral LVs. Rerouting of lymph flow via collateral vessels towards a different LN appears to lead to a recovery of the lymphatic system with sufficient drainage capability, as indicated by decreases in DB and collecting vessel rupture four weeks after ALND-like surgery.

At this point, it is not clear whether the majority of the collateral LVs developed newly or whether they were pre-existing vessels functioning as a 'safeguard' for continuous flow in the case of lymphatic dysfunction. The changes of morphology and pulsing activity indicate that the collateral LVs need to proliferate and mature to fulfil their function. The appearance of collateral vessels already one week after trauma suggests that at least some of these collaterals develop from pre-existing vessels. However, the progressive increase in LVs over the 4 weeks after surgical trauma indicates that also new LVs appear to be formed to enhance lymphatic drainage. Their development and maturation might be stimulated mechanically by an increase in interstitial fluid accumulation, as it has been shown for the development of LVs in the mouse embryo [12]. Artificial increase of interstitial and intralymphatic fluid pressure, i.e. by arm exercise, has also been shown to induce beneficial effects directly after LN surgery [13].

Similar changes of lymphatic anatomy and drainage as described after ALND-like surgery have also been shown in patients with symptomatic lymphedema [5, 6]. This implies that a major trauma such as ALND causes an irreversible destruction of the lymphatic architecture at the surgical site. Shortly after surgery, the development of collateral lymph vessels leads to a recovery of the lymphatic drainage as shown above. However, the function of these newly established lymphatic vessels might be

disturbed by tissue remodelling and associated fibrotic changes, i.e. those induced by radiation therapy which leads to a loss of dermal lymphatic vessels [14, 15], and by inhibition of lymphatic pulsing [16].

Therapeutic approaches should therefore support collateral lymphatic vessel development, i.e. by exercise shortly after surgery or by pharmacological therapy. Radiation therapy should be restricted to the surgical site to protect collateral lymph vessels and to avoid extensive remodelling of the extracellular matrix. Surgical interventions should be performed as minimally invasive as possible to preserve the lymphatic system.

Taken together, this is the first study to reveal changes of lymphatic flow directly after different types of surgical trauma. We found that SLNB-like surgery does not cause an impaired lymphatic drainage in the majority of cases. In contrast, ALND-like surgery resulted in temporary decompensation of the lymphatic system. Rerouting of lymph via collateral vessels to the inguinal LN leads to a recovery of lymphatic function. Our study also indicates that collateral LVs develop and mature, and represent a major pathway for the lymph after extensive trauma. Although these findings were obtained in mouse models, we assume that the reaction of the human lymphatic vasculature to surgical trauma might be similar.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement The experiments were performed in accordance with animal protocols approved by the Kantonales Veterinäramt Zürich.

References

1. McLaughlin SA, Wright MJ, Morris KT, Giron GL, Sampson MR, Brockway JP, Hurley KE, Riedel ER, Van Zee KJ (2008) Prevalence of lymphedema in women with breast cancer 5 years after sentinel lymph node biopsy or axillary dissection: objective measurements. J Clin Oncol 26:5213–5219. doi:10.1200/JCO.2008.16.3725
2. Mansel RE, Fallowfield L, Kissin M, Goyal A, Newcombe RG, Dixon JM, Yiangou C, Horgan K, Bundred N, Monypenny I, England D, Sibbering M, Abdullah TI, Barr L, Chetty U, Sinnett DH, Fleissig A, Clarke D, Ell PJ (2006) Randomized multicenter trial of sentinel node biopsy versus standard axillary treatment in operable breast cancer: the ALMANAC trial. J Natl Cancer Inst 98:599–609. doi:10.1093/jnci/djj158
3. Weissleder H, Weissleder R (1988) Lymphedema: evaluation of qualitative and quantitative lymphoscintigraphy in 238 patients. Radiology 167:729–735
4. Moshiri M, Katz DS, Boris M, Yung E (2002) Using lymphoscintigraphy to evaluate suspected lymphedema of the extremities. AJR Am J Roentgenol 178:405–412. doi:10.2214/ajr.178.2.1780405
5. Rasmussen JC, Tan IC, Marshall MV, Adams KE, Kwon S, Fife CE, Maus EA, Smith LA, Covington KR, Sevick-Muraca EM (2010) Human lymphatic architecture and dynamic transport imaged using near-infrared fluorescence. Transl Oncol 3:362–372
6. Escobar-Prieto A, Gonzalez G, Templeton AW, Cooper BR, Palacios E (1971) Lymphatic channel obstruction. Patterns of altered flow dynamics. Am J Roentgenol Radium Ther Nucl Med 113:366–375
7. Sharma R, Wang W, Rasmussen JC, Joshi A, Houston JP, Adams KE, Cameron A, Ke S, Kwon S, Mawad ME, Sevick-Muraca EM (2007) Quantitative imaging of lymph function. Am J Physiol Heart Circ Physiol 292:H3109–H3118. doi:10.1152/ajpheart.01223.2006
8. Tilney NL (1971) Patterns of lymphatic drainage in the adult laboratory rat. J Anat 109:369–383
9. Proulx ST, Luciani P, Alitalo A, Mumprecht V, Christiansen AJ, Huggenberger R, Leroux JC, Detmar M (2013) Non-invasive dynamic near-infrared imaging and quantification of vascular leakage in vivo. Angiogenesis. doi:10.1007/s10456-013-9332-2
10. Proulx ST, Luciani P, Christiansen A, Karaman S, Blum KS, Rinderknecht M, Leroux JC, Detmar M (2013) Functional near-infrared imaging reveals rerouting of lymphatic tumor drainage after sentinel lymph node metastasis. Biomaterials. doi:10.1016/j.biomaterials.2013.03.034
11. Rasband WS (1997–2011) ImageJ. U. S. National Institutes of Health, Bethesda
12. Planas-Paz L, Strilic B, Goedecke A, Breier G, Fassler R, Lammert E (2012) Mechanoinduction of lymph vessel expansion. EMBO J 31:788–804. doi:10.1038/emboj.2011.456
13. Kilbreath SL, Refshauge KM, Beith JM, Ward LC, Lee M, Simpson JM, Hansen R (2012) Upper limb progressive resistance training and stretching exercises following surgery for early breast cancer: a randomized controlled trial. Breast Cancer Res Treat 133:667–676. doi:10.1007/s10549-012-1964-1
14. Avraham T, Clavin NW, Daluvoy SV, Fernandez J, Soares MA, Cordeiro AP, Mehrara BJ (2009) Fibrosis is a key inhibitor of lymphatic regeneration. Plast Reconstr Surg 124:438–450. doi:10.1097/PRS.0b013e3181adcf4b
15. Avraham T, Yan A, Zampell JC, Daluvoy SV, Haimovitz-Friedman A, Cordeiro AP, Mehrara BJ (2010) Radiation therapy causes loss of dermal lymphatic vessels and interferes with lymphatic function by TGF-beta1-mediated tissue fibrosis. Am J Physiol Cell Physiol 299:C589–C605. doi:10.1152/ajpcell.00535.2009
16. Wang GY, Zhong SZ (1985) Experimental study of lymphatic contractility in lymphedema and its clinical significance. Microsurgery 6:199–203